

CLAIMS

1. A method of imaging a plurality of micro-sample light emitting sites simultaneously onto separately addressable detectors, which may be discrete regions of the array detector, so that light emitted from each site can be monitored by one of the detectors, wherein a corresponding plurality of objective lenses each comprising a micro-lens are located adjacent to the micro-sample array with one objective lens for each micro-sample, the latter are located at or near the focal point of each of the micro-lenses so that light emanating from each micro-sample is collected by its respective objective lens and converted into a beam of parallel or near parallel rays, the objective lenses are arranged so that the axes of all the beams issuing therefrom are parallel and spaced apart, and the beams are focused by a focusing lens through a single point and collected beyond that point by detector lens means which serves to reconstitute the parallel beams for presentation to the detector array, wherein optical discrimination between fluorescence emanating from upstream and downstream regions of the micro-samples is improved by a pinhole aperture located at the focal point of the focusing lens so that light which is not emanating from the focal point of each of the objective lenses adjacent the micro-sample sites will be out of focus at the small aperture pinhole.

2. A method according to claim 1, wherein the axes of the objectives are angled with appropriate adjustment of the optical characteristics of the objectives and/or downstream optical system.

3. A method according to claim 1 or claim 2, wherein a single focusing lens (which may be a multi-component lens) is employed for directing all the beam paths through the single point.

~~4. A method according to any of claims 1 to 3, wherein, the micro-samples are~~
positioned relative to the micro-sample objective lenses so that the region of interest is as
close as possible to the focal point of the respective objective lens.

5. A method according to any of claims 1 to 4, wherein the samples are located on a
planar support with the regions of interest all in the same plane so that the objective
lenses can likewise all be in the same plane parallel to that containing the regions of
interest in the samples.

6. A method according to any of claims 1 to 5, including the step of adjusting the
position of the micro-sample array relative to the lens array and also the step of
individually adjusting the position of at least the objective lenses relative to the micro-
samples or vice versa to ensure that the regions of interest in the micro-samples are at
the focal point of the respective micro-sample objective lenses.

7. A method according to any of claims 1 to 6, wherein in order to provide spectral
separation based on wavelength, a filter is included in the light path either between the
micro-sample objective lenses and the focusing lens means ahead of the pinhole, or
between the detector lens and the detector array.

8. A method according to claim 7, wherein the spectral filter is located in a region in
which the light paths are parallel or nearly parallel.

9. A method according to any of claims 1 to 8, according to which where fluorescence
is the mechanism which generates the radiation which is to be focused onto a detector,
excitation radiation to produce the fluorescence is applied only to a region of interest
within each micro-sample rather than over the whole of the micro-sample.

10. A method according to claim 9, wherein excitation radiation is injected into the
multipath optical system so as to proceed in a parallel sense towards the array of
objective lenses, in an opposite sense to the light which emanates from the micro-

samples, so as to be focused by the objective lenses onto the region of interest in each micro-sample.

11. A method according to claim 10, wherein the excitation radiation is injected as a parallel beam into the optical path, at right angles thereto, and a 45° beam splitting device is provided onto which the parallel excitation radiation is incident and from which it is directed in a parallel manner towards the micro-sample imaging lenses, but through which radiation from the micro-samples can pass to the focusing lens.

12. A method according to any of claims 1 to 11, wherein excitation radiation is produced using a laser such as an argon ion laser, and a beam expander is employed to expand the cross-section of the laser beam into a relatively large area beam equivalent to the area of the parallel array of micro-sample objective lenses.

13. A method according to any of claims 1 to 12, having an 8 x 12 array of micro-sample objective lenses on the same 8 x 12 matrix as a standard 96 well plate, and if the imaging system is to be used to inspect for example a 384 well plate, the latter is mounted on an XY stage so that it can be moved relative to the array of objective lenses in manner known per se so as to present groups of 96 wells making up the 384 wells, to the 96 lens array.

14. A method according to any of claims 1 to 13, wherein the parallel beams of light directed towards the detector array are transferred to the said array via optical fibres, in the form of a fibre optic bundle or fibre optic plate.

15. A method according to any of claims 1 to 14, wherein the detector array is a charge coupled device having a large number of separately addressable regions (each of which is commonly referred to as a pixel) and groups of adjacent pixels (or individual pixels) from the detector for each sample are addressed, to enable good resolution to be obtained in the XY sense as between the light from one sample and another.

~~16. A method according to claim 15, wherein the charge coupled device is cooled, for example cryogenically.~~

17. A method according to any of claims 1 to 14, wherein the detector array comprises an array of photomultipliers, one photomultiplier for each of the channels (optical paths).

18. A method according to claim 17, wherein optical fibres or bundles forming cables are employed to convey the light from each of the apertures in a mask to the windows of photo-multipliers which together occupy an area considerably greater than that of the mask.

19. A method according to claim 17 or 18, wherein the photomultipliers are replaced by an image intensifier or an intensified CCD.

20. A method according to any of claims 17 to 19, wherein the photomultiplier tubes, image intensifiers and intensified CCD arrangements are gated electronically so as to enable the delays and short integration periods to be generated as required by time resolved fluorescence or luminescence applications.

21. A method according to any of claims 1 to 20, wherein further lenses for focusing the parallel beams of light directed towards the detector array are employed to improve resolution at the detector surface either in combination with a fibre optic transfer bundle, or otherwise.

22. A method according to any of claims 1 to 21, wherein micro lenses, optionally in combination with a fibre optic transfer plate are employed in the objective lenses adjacent the micro-samples.

23. A method according to claim 22, in which micro lenses have one infinite conjugate.

~~24. A method according to any of claims 1 to 23, wherein where a filter is located~~
ahead of the detector, apertured masks are placed on either side of the filter to collimate
the parallel beam to further reduce background and cross-talk.

25. Apparatus adapted for performing the method of any of claims 1 to 24, comprising
means for supporting a micro-sample array on a substrate in close proximity but parallel
to an array of micro lenses arranged so as to correspond on a one to one basis with the
positions and spacing of at least some of the micro-samples on the substrate, each of the
micro lenses being positioned relative to a region of its related micro-sample by a
distance equal to the focal length of the lens so that light emitted from that region of the
micro-sample will emerge from the lens as a parallel beam parallel to the axis of the
lens, and the parallel beams of light are focused by means of a single focusing lens
through a pinhole aperture onto a detector lens so as to produce an image of the micro-
sample light emissions in the plane of an array of individually addressable photoelectric
detectors, such as regions of an addressable CCD array, and circuit means is provided to
which signals read out from the array are supplied in the form of a sequence of digital
values or otherwise, each corresponding to the light incident on a region of the detector
for a given period of time from one of the micro-samples, and computing and analysing
circuit means is provided, responsive to the electrical signals, together with memory
means for storing data indicative of the light found to be emitted from each of the micro-
samples, for storing those values together with address information, whereby each stored
value can be identified with the micro-sample on the substrate from which the light
giving that value has been emitted by reference to the position of the region in the
detector array and by correlating the position of the sample in the sample array.

26. Apparatus according to claim 25, further including a beam splitter, such as a
dichroic mirror, interposed in the optical path between the micro lenses and the focusing
lens to enable on the one hand light to pass from the lenses to the focusing lens, and on
the other hand to enable excitation radiation, typically light of a particular wavelength, to
be reflected as a parallel beam towards the micro lenses, thereby utilising the optical
focusing characteristics of the micro lenses to focus the parallel light into spots of light

~~which register with the micro-samples so that the latter are individually radiated by~~
excitation light which is predominantly incident on that region of each micro-sample
which is to be inspected for fluorescence after the excitation radiation has been removed,
and filter means provided in the optical path between the beam splitter and the detector
array to generally attenuate any excitation wavelength radiation travelling towards the
detector and generally prevent such radiation from reaching the detector.

27. Apparatus according to claim 25 or claim 26, including a laser source as the source
of excitation radiation a beam expander for enlarging the cross-section of the laser beam
and presenting a generally uniform parallel beam of excitation radiation for entry into the
imaging system via the beam splitter or dichroic mirror.

28. Apparatus according to any of claims 25 to 27, including shutter means to inhibit
the passage of the source light, except when required for excitation purposes, and further
shutter means synchronised with that associated with the source to prevent light of any
wavelength reaching the detector whilst excitation light is projected into the system.

29. A method of analysing fluorescence emitted by radiation excited samples in an array
of samples comprising the steps of focusing light emitted from each said sample at
infinity so as to form a parallel beam, in parallel with the light from all of the other
sample sites making up the array, subsequently focusing all the parallel light paths
through a single point and locating at the point a small pinhole aperture to restrict
unwanted light from fluorescing material upstream and downstream of the sites of
interest in the samples, and re-establishing a parallel array of light beams by the use of a
further lens so as to present to an addressable detector array a plurality of parallel light
paths corresponding to the light paths from the samples, and individually addressing
different regions of the detector array onto which the parallel light paths impinge, to
determine the light incident thereon, and storing data relating to the quantity of incident
light on each said region of the detector array together with address information to
enable the data to be reconciled with the position of the sample in the array on the
substrate to which that data relates.

~~30. The method according to claim 29, further comprising the step of introducing~~
periodically into the optical system excitation wavelength illumination and projecting same
through the optical imaging devices associated with the array of samples to project the
excitation illumination onto a specific region in each said sample, thereafter extinguishing
the excitation wavelength light and enabling fluorescence caused by the excitation to pass
through the same optical devices to emerge as parallel rays of light for transfer to a
detector, for analysis as above mentioned.

31. The method according to claim 29 or claim 30, wherein the objectives are arranged
above or below a sample array.

32. The method according to any of claims 29 to 31, when used to perform immediate
fluorescence analysis or time resolved fluorescence analysis in which a shutter is provided
to inhibit the transfer of light to the detector, excitation radiation is supplied for a short
interval of time and then shut off (either by pulsing the source out using further shutter or
both), after a selected interval of time the shutter preventing transfer of light to the detector
is opened and after an appropriate integration interval (which can be very short or longer
as described) the residual charge pattern on the charge coupled detector array is
interrogated to generate a signal relating to the charge pattern (and therefore indicative of
light incident thereon) for processing and storage as aforesaid.